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Surface modification of Polydimethylsiloxane by hydrogels for microfluidic applications

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Abstract: *In vitro*, hydrogel-based ECMs for functionalizing surfaces of various material have played an essential role in mimicking native tissue matrix. Polydimethylsiloxane (PDMS) is widely used to build microfluidic or organ-on-chip devices compatible with cells due to its easy handling in cast replication. Despite such advantages, the limitation of PDMS is its hydrophobic surface property. To improve wettability of PDMS-based devices, alginate, a naturally derived polysaccharide, was covalently bound to the PDMS surface. This alginate then crosslinked further hydrogel onto the PDMS surface in desired layer thickness. Hydrogel-modified PDMS was used for coating a topography chip system and in vitro investigation of cell growth on the surfaces. Moreover, such hydrophilic hydrogel-coated PDMS is utilized in a microfluidic device to prevent unspecific absorption of organic solutions. Hence, in both exemplary studies, PDMS surface properties were modified leading to improved devices.

Keywords: PDMS, alginate, hydrogel, microfluidic chips, cell culture.

1 Introduction

In recent years, a range of biomaterials have been developed for cell culture systems that provide ways to control mechanical, compositional, and structural cues with the aim to represent native tissues [1]. With the emergence of microscale biotechnology, such as microfluidic-based tissue and organ chips, human cells are cultured in tissue-specific three-dimensional settings. These platforms are designed to recapitulate the multifaceted cellular and extracellular properties, so that a minimally functional unit of human physiology is established in a controlled and straightforward manner [2]. Moreover, topography, especially smoothly curved features, is another relevant parameter in mimicking the native cellular environment. Topographies are omnipresent in situ and are such artificial surface features are important in cell research and development of improved implant surfaces [3]. To fabricate either microfluidic platforms or cell culture substrates with topography in lab environments, one of the most frequently used materials is poly(dimethylsiloxane) (PDMS). The advantages of PDMS are its biocompatibility, optical transparency, and elasticity. However, the extremely hydrophobic nature of PDMS often limits its applicability. It can lead, for example, to a significant nonselective adsorption of hydrophobic molecules, including oxygen and many drug compounds [4]. Therefore, various methods have been proposed to modify the PDMS surface to impart hydrophilicity ranging from oxygen plasma treatment to chemical modifications [5].

In this work, we validated in two application examples an effective PDMS coating with alginate, which is a polysaccharide derived from brown algae. First, a monolayer of alginate was covalently linked to the PDMS surface. This layer acted as a "glue" to allow the strong, permanent adhesion of further hydrogel onto the PDMS surface [6]. We applied two different molding procedures to generate alginate layer onto curvature microstructures. The resulting hydrogel-coated PDMS substrate was used as an *in vitro* cell culture platform to study cellular behaviour in response to topographic and mechanical stiffness. In the second application example, the surface modification protocol was used to modify a PDMS microfluidic chip device with a hydrophilic layer to avoid hydrophobic adsorption of 2,6-Diisopropylphenol (Propofol) on the PDMS.

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2 Materials and method

2.1 Materials

PDMS (10:1, curing agent:base, Sylgard®184 Silicone Elastomer Kit, Dow Corning) repetitive molding was fabricated as described in our previous work [3]. A Photo-resist AZ® 40XT (MircoChemicals GmbH, Ulm, Germany) was utilized to fabricate a chip with curvature structures. This combined photolithography with a thermal reflow process [3].
PDMS microfluidic chip: For coating alginate on microfluidic chip surfaces, PDMS was poured into a 3D printed master with the negative chip structures, having channels of 500×20 µm (width × thickness) as well as reservoir and connection units. Upper and lower parts of the chip were cured at 60 °C for 2h. Both parts were precisely assembled under a stereomicroscope, and the PDMS chip was fully cured at 60 °C for overnight.

2.2 Alginate coating and molding of curvature structures

An alginate monolayer was first covalently bound on the PDMS surface to obtain a monolayer alginate-PDMS, as described in [5]. The alginate-PDMS was further treated by different coating methods. For the coating procedure of PDMS surfaces with curved topographies, the second mold of the concave structure was first modified by an alginate monolayer. Then the surface was rinsed three times by $CaCl_2$ (0.1 mg/ml) solution. The rest of the $CaCl_2$ solution was dried in an oven at 60 °C for 30 min. 1% alginate solution was poured on the Ca^{2+}-ion pretreated surface for overnight. Finally, the sample was washed thoroughly with water and stored in $CaCl_2$ solution.
For the molding procedure using the alginate hydrogel a flat PDMS substrate was first coated by an alginate monolayer. About 1 mm thick 1% alginate solution was poured on the alginate-PDMS surface. Alternatively, a covalent cross-linker adipic acid dihydrazide (AAD) was utilized. The molar ratio of sodium alginate monomer, AAD, EDC, and NHS were mixed as 10:1:4:4. Meanwhile, the first mold of convex structure was treated by oxygen plasma for 30 s. It was carefully attached to the alginate solution on the flat PDMS. As for the ion crosslinking, $CaCl_2$ solution was then slowly dropped by the side of the flat PDMS substrate. The sample was stored overnight for cross-linking. For the covalent crosslinking with AAD, the sample was incubated at 37 ° C for 1 hour. Finally, the convex PDMS mold was peeled off from the alginate surface.

2.3 Alginate coating on PDMS microfluidic chip surfaces

The microfluidic chip was connected with a microfluidic pump system (ibidi pump system, ibidi GmbH, Martinsried, Germany). The flow rate was set for the following reactions as 2.7 ml/min. First, 1 M NaOH was flowed through the channels for 1 h to induce hydroxyl groups. Subsequently, APTES was incubated in the channel for one hour. In order to get a monolayer alginate-coated PDMS microchannel, the premixed 1% alginate solution (molar ratio of alginate: Sulfo-NHS: EDC = 1: 12: 10) was flowed in and incubated in the channel for 24 hours. Between each step, the substrate was thoroughly rinsed with water.

2.4 Surface characterization

Contact angle was measured by a drop shape analyser (DSA10-MK2, Krüss GmbH, Hamburg, Germany). Contact angle ($\theta = mean \pm SD, N \geq 5$) was calculated by the angle between the drop shape function and the sample surface.
Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy (ATR-FTIR): IR spectra were scanned from 4000 to 650 cm^{-1} in an ATR mode (Frontier™ FT-IR Spectrometer, Perkin Elmer Inc., Massachusetts, USA). The samples were dried and measured under the same pressure.
Gas chromatography-mass spectrometry (GC-MS): Samples were collected in a glass tube with rubber septum. A Solid-phase microextraction (SPME) fibre (PDMS/DVB, SUPELCO®, Sigma Aldrich) was pricked through the septum for the partitioning of the analytes. The SPME fibre was exposed in analytes solution for 30 s. After sample adsorption, the SPME fibre was introduced into the 7820A GC System with a 5977 B quadrupole mass spectrometer (MSD, Agilent, Santa Clara, United States), where the adsorbed analytes were thermally desorbed and delivered to a GC column. The temperature of the split/splitless inlet was 250 °C, and the split ratio was 1:10. The thermal conditions for the MSD were quadrupole temperature 250 °C, and ion source temperature 250 °C. Mass spectra and reconstructed chromatograms (total ion current, TIC) were obtained by automatic scanning in the mass range m/z 50–300. The GC–MS data were acquired after a solvent delay of 4 min. Identification of propofol (5 µg/ml in Dulbecco's modified Eagle medium (DMEM) with 2% fetal calf serum (FCS)) was carried out by comparison of retention times and mass spectra of standard in a NIST 14 spectral library.
Cryogenic scanning electron microscopy (CryoSEM): Samples were frozen in liquid nitrogen. The following steps were performed in a Freeze-Fracture system (Leica EM BAF

060, Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany) under vacuum. The temperature was stepwise increased to 183 K. The samples were sublimated for 1 h and sputtered with 4.2 nm platinum (1.55 kV. 0.55 mA). They were transferred in a Zeiss Ultra 55 scanning electron microscope for imaging using an accelerating voltage of 2 keV.

2.5 Cell culture

Mouse fibroblast NIH/3T3 cells (CRL-1658™, ATCC®, Manassas, USA) were cultured in DMEM, supplemented with 10% FCS, with penicillin and streptomycin in a humidified incubator (Heraeus, Thermo Fisher Scientific Inc., Germany) at 37 °C and 5% CO_2. The sample surface was first disinfected with 70% EtOH, then rinsed 3 times with PBS. It was either plasma treated or incubated with 50 µg/ml Collagen for 10 min. After rinsing 3 times with PBS, cells were seeded in a six-well plate on the alginate-coated samples and allowed to adhere overnight. The cell density was kept constant at 400,000 cells/ml in the whole study unless otherwise stated.

Light microscopy: Live cell imaging was performed 24 and 48 h after seeding by using a Zeiss Observer Z1 microscope (Zeiss, Germany). Phase contrast images were acquired by an A-Plan 10×, 0.25 Ph1 objective.

3 Results

Surface modification after alginate hydrogel coating was characterized by various methods. As shown in Figure 1A and B, the contact angle of a water droplet on PDMS and alginate-PDMS surface changed dramatically from $\theta_{PDMS} = (110.2 \pm 1.7)°$ to $\theta_{alginate-PDMS} = (19.4 \pm 2.8)°$. The hydrophobic surface property of PDMS was altered by the alginate monolayer to be hydrophilic. ATR-FTIR spectra from different sample preparations (Figure 1C, black, red and green lines) demonstrate the characteristic absorption bands of alginate polysaccharide structure, symmetric 1414 cm^{-1} and asymmetric 1600 cm^{-1} (C–O stretching) from the carboxylate salt groups. Conversely, the bands at 1126 cm^{-1} (C–C stretching), 1021 cm^{-1} (C–O–C stretching) and 947 cm^{-1} (C–O stretching) are obtained from the pure PDMS sample (Figure 1C, blue line) [6, 7]. The analysis further demonstrates the successful immobilization of the hydrogel on the PDMS. Figure 1D presents a cryo-SEM image of monolayer alginate-PDMS after the coating procedure on curved PDMS structures. Figure 1E presents a thick alginate layer after the molding procedure on flat PDMS substrates. The thin initial coating shows no prominent structure, whereas the thick layer of alginate in Figure 1E has a typical hydrogel surface.

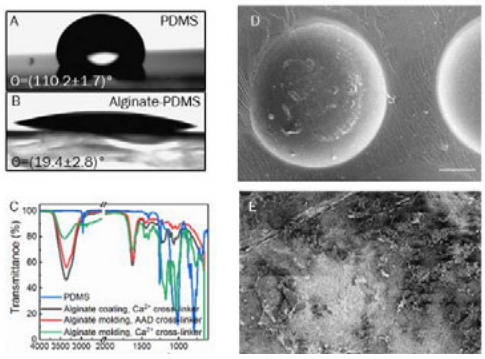

Figure 1. Surface characterization of alginate coated PDMS. A and B are the contact angle of water droplets on the PDMS and alginate-PDMS surface. $\theta_{PDMS} = (110.2 \pm 1.7)°$ and $\theta_{alginate-PDMS} = (19.4 \pm 2.8)°$. C represents the FTIR spectra of pure PDMS and various alginate coated PDMS surface using the Ca^{2+} (black and green lines) and AAD (red lines) cross-linkers. D and E are Cryo-SEM images of alginate-PDMS on the curvature structures and alginate coating after molding procedure, correspondingly. Scale bar: 100 µm.

Next, cells were grown on the structures, as shown in Figure 2A-E. Only few cells were sitting on the plasma-treated PDMS with semi-spherical structures (48 h). Conversely, cells grew on the collagen-coated PDMS curvature structure to high density (Figure 2B). Figure 2D-E represent experiments with alginate-coated curvature surface using the three different procedures, correspondingly, the molding procedure AAD cross-linker (C), the molding procedure Ca^{2+} cross-linker (D), and the coating procedure Ca^{2+} cross-linker (E).

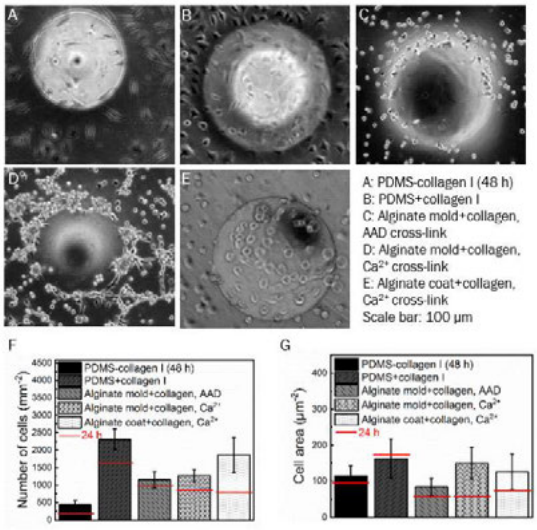

Figure 2. Mouse fibroblast cell culture after 48 h on variant alginate coated curvature structures. A-E are phase contrast images of mouse fibroblast cells on the curvature structures. F represents the number of cells on the curvature structure. And G shows the cell area on the curvature structure. The errorbars demostrate the standard deviation of each sample for N>30 cells.

After 48 h, cells grew on the alginate-coated curvature surface. The morphology of the fibroblast cells shows a stretched and elongated shape on the collagen PDMS surface, in comparison to the alginate-coated PDMS. Number of cells on the curvature structure was counted at 24 h and 48 h (Figure 2F). The red lines indicate the mean values at 24 h. Cells proliferated under all conditions. The Ca^{2+} cross-linked alginate procedure shows the highest relative increase of the proliferation (Figure 2F, points patterned white column). Figure 2G shows the calculated average cell area. Red lines indicate the mean values at 24 h. Cells on both PDMS (with or w/o collagen coating) surfaces have a nearly constant morphology. Conversely, cells on the alginate coated surface increased their area between 24h and 48 h. Cells growing on Ca^{2+} cross-linked alginate (molding procedure) show the highest increase in number (Figure 2F, grid-patterned grey column).

In the second application, the alginate coating procedure was validated in a microfluidic chip system to inhibit the unspecific adsorption of propofol in PDMS. 5 µg/ml propofol solution flowed through the microfluidic system. Propofol concentration were measured for a) w/o a chip, b) pure PDMS chip, and c) a PDMS chip coated with alginate monolayer. Exemplary chromatographs are given in Figure 3A. The propofol peak was pointed out by a black arrow. The maximum counts of the propofol at 14.3 min is approximately 10-fold higher for the alginate-coated chip than for the uncoated PDMS system. Figure 3B is a detailed mass spectrum of propofol. Figure 3C and D are extracted ion chromatograms, correspondingly m/z 178 and m/z 163, from experiments with uncoated (in black) and alginate-coated (in red) PDMS samples. The integrated area of the mass to charge m/z 178 increased by 4.9 folds, as for the m/z 163 by 2.4 folds.

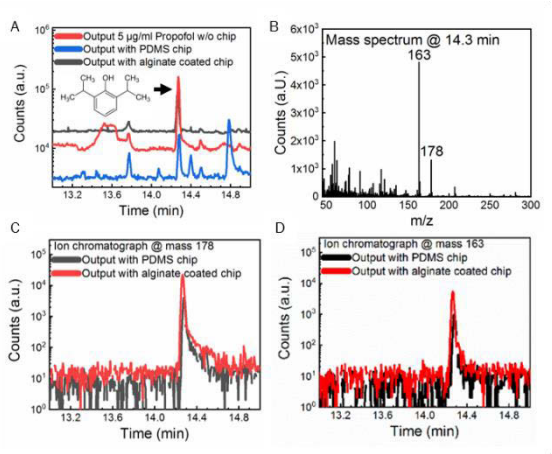

Figure 3. GC-MS of alginate coated microchip. A represents the chromatographs of flow system w/o chip (in red), pure PDMS chip (blue), and a PDMS chip coated with alginate monolayer (in black). B is a detailed mass spectrum of propofol peak at 14.3 min. C and D are extracted ion chromatograms, correspondingly m/z 178 and m/z 163, from uncoated (in black) and alginate coated (in red) samples.

4 Conclusions

We successfully established two procedures to stably coat alginate-hydrogel on PDMS chips with 3D curved features and in a PDMS-based microfluidic chip system. Surface characterization showed the hydrophilic property of the coating. First *in vitro* investigation with NIH3TE cells demonstrated cell adhesion and possibly cell responses to the decreased surface stiffness. Moreover, the alginate-coating of PDMS surfaces in an exemplary microfluidic device could prevent unspecific absorption of propofol, a typical problem of using silicon elastomers in such applications. Therefore, alginate-coating of microfluidic devices used with solutions of highly-absorbable organic compounds could be an appropriate and promising approach.

Author Statement

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